VETERINARSKI ARHIV 93 (1), 117-128, 2023 DOI: 10.24099/vet.arhiv.1502

In vitro and *in vivo* anti-inflammatory, antibacterial and pharmacokinetic properties of baicalein

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VARIA R. D., J. H. PATEL, F. D. MODI, P. D. VIHOL, S. K. BHAVSAR: *In vitro* and *in vivo* anti-inflammatory, antibacterial and pharmacokinetic properties of baicalein. Vet. arhiv 93, 117-128 2023.

ABSTRACT

Baicalein is a bioactive flavone originally isolated from the roots of *Scutellaria baicalensis, Scutellariala teriflora* and *Oroxylum indicum*. The *in vitro* and *in vivo* anti-inflammatory and antibacterial properties of baicalein and pharmacokinetics after its single intramuscular administration were studied in Wistar rats. The i*n vitro* anti-inflammatory activity of baicalein (10, 50 and 100 µM) was tested for its ability to inhibit the COX-2 enzyme by measuring PGE_2 levels and determination of nitric oxide (NO) production in lipopolysaccharide (LPS) treated RAW 264.7 macrophage cells, in which baicalein was found to have significant inhibition of NO and PGE_2 production in RAW 264.7 macrophage cells as compared with the LPS control group. The *in vivo* anti-inflammatory activity of baicalein (200 mg/kg) was assessed using the carrageenan-induced rat paw oedema model, following intramuscular injection. A significant percentage of inhibition of oedema volume was observed when compared with the carrageenan control group. *In vitro* and *in vivo* antibacterial activities of baicalein were determined by the micro broth dilution technique and neutropenic rat thigh infection model, wherein baicalein did not show any antibacterial property. Concentrations of baicalein were determined in rat plasma by high performance liquid chromatography (HPLC) after a single intramuscular administration at a dose of 200 mg/kg body weight, in which the mean peak plasma drug concentration (C_{max}) of 0.77 ± 0.02 µg/mL was achieved at 0.08 h. The mean elimination half-life ($t_{½\beta}$), the apparent volume of distribution ($Vd_{(area)}$), total body clearance ($Cl_{(B)}$) and mean residence time (MRT) were observed as 0.63 ± 0.06 h, 601.03 ± 28.18 L/kg, 677.39 ± 35.36 L/h/kg and 0.76 ± 0.06 h, respectively. Conclusively, in the present study, baicalein did not show *in vitro* or *in vivo* antibacterial property, but proved to have good anti-inflammatory activity. The available anti-inflammatory drugs have proved to have side effects in veterinary and human therapeutics. In this situation, baicalein may become an effective alternative to non-steroidal anti-inflammatory drugs and should also be studied in target animal species. Further research should be carried out to improve the solubility and bioavailability of baicalein through injectable routes.

Key words: antibacterial; anti-inflammatory; baicalein; pharmacokinetic; rat

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ISSN 0372-5480
Printed in Croatia

Introduction

In veterinary and human medicine, allopathic drugs have been shown to cause many side effects and most antibiotics have developed resistance while otherwise effective at their usual dosage. Phytochemicals are derived from different parts of medicinal plants and can be used as solo agents for therapeutics or together with routine allopathic drugs. Phytochemicals may enhance the efficacy of allopathic drugs and in that way they decrease the side effects by lowering the dosage of drugs that have a lower margin of safety. In this context, ethnoveterinary practices are an effective alternative to antibiotics and other drugs in livestock management (BALAKRISHNAN et al., 2017). Of all the phytochemicals, flavonoids have attracted most attention for research and the development of new molecules. Flavonoids comprise one of the largest and most widely distributed groups of secondary plant metabolites (ROBARDS and ANTOLOVICH, 1997) and they have been identified as a good alternative to combat multi drug resistance in the treatment of infectious diseases and are available in variety of dietary sources (RAGUNATHAN and RAVI, 2015). Baicalein is a flavonoid and has shown multiple pharmacological activities *via in vitro* experimentation using crude extracts and isolated phytochemicals (HE et al., 2015). Regarding the potential of the phytochemical baicalein, bioprospecting of anti-inflammatory and antimicrobial activities in animal models is the need of the hour. In addition, knowledge of pharmacokinetics is equally important to understand the movement of drug molecules in the body. Phytochemicals, and particularly flavonoids, are administered orally in therapeutics and very few studies are available on injectable baicalein preparations in animals and humans. In the present study, an attempt was made to make injectable baicalein that can serve its purpose in therapy without adverse effects, and can be administered concurrent to the available synthetic antibacterial and anti-inflammatory compounds. With this in mind, the present study was carried out to explore the *in vitro* and *in vivo* antibacterial and anti-inflammatory activities of baicalein, as well as to determine its pharmacokinetic behaviour following intramuscular administration in Wistar rats.

Materials and methods

Experimental animals: Female albino Wistar rats (n=24) weighing 305 ± 2.60 grams were used to evaluate the *in vivo* anti-inflammatory properties. After a 15 day washout period, some animals were used, weighing 353 ± 4.81 grams, to evaluate *in vivo* antibacterial activity, and another 30 animals, weighing 234 ± 8.81 grams, were used for pharmacokinetic study. The experimental protocols were approved by the IAEC of Veterinary College, NAU, Navsari with permission numbers 057-VCN-VPT-2018 and 058-VCN-VPT-2018.

Reagents: The pure form of baicalein (98%), Nω-Nitro-L-arginine methyl ester hydrochloride (NAME), lipopolysaccharide (LPS), Dulbecco's modified eagle's medium – high glucose (DMEM), 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), iodonitrotetrazolium chloride (INT), lambda (λ) carrageenan and cyclophosphamide were purchased from Sigma-Aldrich, St. Louis, USA. Indomethacin was procured from Calbiochem, USA. Triethanolamine was purchased from MP Biomedicals, USA. Dimethylsulfoxide (DMSO), PEG-200, acetonitrile (HPLC grade), glacial acetic acid, sodium nitrite, 25% ammonia solution and 1-methyl-2 pyrrolidone were purchased from Merck Specialties Private Limited, Mumbai. Celecoxib, chloramphenicol, sulfanilamide, eosin methylene blue (EMB) agar, N-(1-Naphthyl) ethylenediamine dihydrochloride (NEDD), brain heart infusion (BHI) broth, antibiotic antimycotic solution 100X liquid and foetal bovine serum were purchased from Himedia Laboratories Private Limited, Mumbai. Bacterial cultures were obtained from the national collection of industrial microorganisms, Pune and murine macrophage cell line RAW 264.7 was purchased from the national centre for cell science, Pune. HPLC grade water was used in all *in vitro* and *in vivo* protocols. The Prostaglandin E_2 express ELISA kit was obtained from the Cayman Chemical Company, USA.

In vitro anti-inflammatory effect: The *in vitro* anti-inflammatory effect of baicalein was studied

in the murine macrophage cell line RAW 264.7 by determining COX-2 enzyme inhibition via detection of PGE_2 concentration and measurement of NO production inhibition. Baicalein (100 µM, 50 µM and 10 µM) was prepared using triethanolamine: DMSO: cell culture medium in a ratio of 0.2:0.2:99.6. Macrophage cells were treated according to the protocol given by VARIA et al. (2020). The cells were supplemented with 1600 µL fresh cell culture medium and 200 µL baicalein in different concentrations (100 µM, 50 µM and 10 µM) for NO production inhibition and PGE_2 inhibition assay. In the positive control wells, instead of baicalein, Nω-Nitro-L-arginine methyl ester hydrochloride (NAME, 100 µM) and celecoxib (100 µM) were used for NO production inhibition assay and PGE_2 inhibition assay, respectively. Vehicle control wells were treated with 1600 µL cell culture medium and 200 µL of the vehicle in which the baicalein was prepared (triethanolamine: DMSO: cell culture medium in 0.2:0.2:99.6 ratio), and 1800 µL cell culture medium was added to the LPS control wells. The assay was performed in triplicate. All plates were incubated at 37°C and 5% CO_2 in a humidified condition for two hours, after which 200 µL LPS (1 µg/ml) was added to all the wells, and they were incubated again for twenty-four hours. After incubation, the supernatant was collected which was used to quantify the COX-2 enzyme by measuring the PGE_2 concentration using a prostaglandin E_2 express ELISA kit (BARTON et al., 2014), and to quantify nitrite accumulated in the medium as an indicator of NO production using a Griess reaction (CHOI et al., 2018). The concentration of NO was estimated using a sodium nitrite standard calibration curve (1.56 µM to 50.0 µM) correlation equation. The results were expressed as the percentage inhibition of PGE_2 and NO production compared with the LPS control.

Cell viability: Cell viability was tested in the samples by MTT assay (CHOI et al., 2018). Twenty µL of MTT solution (5 mg/ml) was added to each well after sample collection for assay and the plates were incubated for 4 h at 37°C. The supernatants were collected and the formazan crystals formed in the wells were dissolved in 200 µL of DMSO for 30 minutes at 37°C. The optical density was read using a spectrophotometer at 570 nm. The cell viability percentage was calculated.

In vivo anti-inflammatory efficacy: The carrageenan-induced rat paw oedema model was used as described by SUEBSASANA et al. (2009) and MODI et al. (2019), with minor modifications. Baicalein (200 mg/kg) was prepared in DMSO: PEG-200:1-methyl-2 pyrrolidone in a ratio of 4.5: 4.5: 1.0. The experimental animals (n=24) were divided into four groups with six animals in each group, *viz*. the carrageenan control, the vehicle control (200 µl baicalein vehicle (DMSO: PEG-200: 1-methyl-2 pyrrolidone in a ratio of 4.5: 4.5: 1.0) IM), the positive control (indomethacin 5 mg/kg IM) and the test group (baicalein 200 mg/kg IM). A mark was made on the left hind paw of each animal and the initial volume was measured using a plethysmometer. Thereafter, 100 µl lambda carrageenan (1%) was injected subcutaneously into the sub-plantar region of the left hind paw. The test drug and the positive control drug were injected half an hour before carrageenan administration. Paw volumes were measured before carrageenan administration and at 1, 2, 3, 4, 5 and 6 h after carrageenan administration. The results were expressed as percentage oedema formation in relation to the initial paw volume, and percentage inhibition of oedema formation in comparison to carrageenan control group.

In vitro antibacterial effect: Baicalein stock (20 mg/mL) was prepared in a one percent ammonia solution. After overnight incubation, all bacterial cultures were prepared to the McFarland 0.5 standard, equivalent to 1.5×10^8 cfu/mL. The final dispensing inoculums were prepared in sterile test tubes by diluting the stock 100 times. Minimum inhibitory concentrations of baicalein were determined for different organisms, such as: *Bacillus subtillis* (ATCC9372), *Streptococcus pyogenus* (ATCC8668), *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922), *Pseudomonas aerugonosa* (ATCC27853), *Proteus mirabilis* (NCIM2241) and *Salmonella typhimurium* (ATCC23564) by the microbroth dilution technique (WIEGAND et al., 2008; VARIA et al., 2020). The visual viability of the bacteria was observed using iodonitrotetrazolium

chloride (INT) dye. This protocol was performed in triplicate.

In vivo antibacterial efficacy: The *in vivo* antibacterial efficacy of baicalein was studied using a neutropenic rat thigh infection model (ZHAO et al., 2016; VARIA et al., 2020). In total, 24 animals were used in this protocol and divided equally into four groups (n=6). Cyclophosphamide was administered to create a neutropenic condition in rats by the intraperitoneal route on day one (150 mg/kg) and on day four (100 mg/kg). The infection was created by means of a 0.2 ml bacterial suspension of *Escherichia coli* (1.5×10^8 cfu/ml) injected into the left thigh on the same day after confirmation of a neutropenic condition in all animals. Baicalein (200 mg/kg) was prepared in DMSO: PEG-200: 1-methyl-2 pyrrolidone in a ratio of 4.5: 4.5: 1.0. The total volume of 200 µl was injected intramuscularly at 2 h and 8 h post infection into the right thigh, and the bacterial suspension (0.2 ml, IM) into the left thigh (group-I). The bacterial suspension (0.2 ml, IM) and chloramphenicol (50 mg/kg, IM) were administered in group-II (positive control), group-III animals were administered the bacterial suspension (0.2 ml, IM) and the vehicle (DMSO: PEG-200: 1-methyl-2 pyrrolidone in a ratio of 4.5: 4.5: 1.0) (0.2 ml, IM) (vehicle control), the growth control animals (group-IV) were treated with the bacterial suspension alone (0.2 ml, IM). After twenty-four hours, the animals were euthanized and one gram thigh muscle samples from the infected site were collected under sterile conditions. Suitable dilutions of samples were prepared, inoculated onto eosin methylene blue (EMB) agar plates, and incubated overnight at 37°C. Bacterial colonies were counted and the $\log_{10}$cfu/gram calculated.

Plasma drug concentrations and pharmacokinetic analysis: The animals (n=30) were divided into six groups. A single dose of baicalein (200 mg/kg dissolved in 1-methyl-2 pyrrolidone) was administered by the intramuscular route to the rats. Blood samples (250 µl) were collected from the experimental animals in K3 EDTA vials, at different time intervals i.e.: 0 (before drug administration), 0.03 (2 min), 0.08 (5 min), 0.167 (10 min), 0.25 (15 min), 0.5 (30 min), 0.75 (45 min), and after 1, 2, 4, 6, 8 and 12 hours, from the retro orbital plexus under light anaesthesia using isoflurane. Multiple rats were used for serial collection of blood at alternating time points. After blood collection, the plasma was separated. Glacial acetic acid (10%) in acetonitrile was added to the plasma samples at 2:1 ratio to precipitate plasma protein, and mixed using a vortex mixer for one minute, followed by centrifugation for 15 minutes at 8000 rpm at 4°C in a refrigerated centrifuge. The clean supernatants were transferred into automatic sampler vial inserts, and 20 µl of the supernatant from them was injected into the HPLC system. A high performance Shimadzu liquid chromatography instrument comprising a binary gradient delivery pump (model LC - 20AP) and UV detector (model CBM-20A), was used for the assay. A reverse phase C_{18} column (ODS; 250 ´ 4.6 mm ID) was used to perform chromatographic separation at room temperature. The mobile phase consisted of a mixture of 1% glacial acetic acid and acetonitrile (50:50 v/v), and the flow rate was 1.0 ml/min at ambient temperature (XING et al., 2006). The effluent was monitored at a wavelength of 275 nm. PK Solutions software (Version 2.0) was used to integrate HPLC data. PK solutions is a well-known automated Excel based program that performs pharmacokinetic data analysis of concentration-time data from biological samples (blood, serum, plasma, lymph, etc.) following administration of a drug, using a non-compartmental model. Baicalein standards in the mobile phase and in drug free rat plasma were prepared as sample preparations. The precision and accuracy of the assay were assessed in concurrence with the linearity study, including the means and coefficients of variance (C.V.) for concentrations of 6.25, 0.78 and 0.098 µg/ml. Recovery in plasma at all concentrations studied was more than 85%, and the C.V. percentages were less than 4%.

Statistical analysis: The data in the present study are depicted as Mean ± S.E. Analysis of the data was performed by one way ANOVA, and the significance level was checked using SPSS-20 software by Duncan's New Multiple Range Test (DNMRT).

Results and Discussion

Anti-inflammatory effect: The percentage inhibition of NO and PGE_2 production at different concentrations were observed with significant differences at all concentrations in comparison to the LPS control (Table 1). Cell viability percentages were checked and compared with the blank control and more than 96 % viability was observed. In the *in vivo* experiment, percentage increases in oedema volume were significantly lower in comparison with the carrageenan control group following administration of baicalein during the study period (Fig. 1). In addition, following intramuscular administration of baicalein, a significant increases in the inhibition percentage of paw volume were observed from 1 to 6 hours (Table 2).

Table 1. *In vitro* percentage inhibition of NO and PGE_2 production in LPS induced RAW 264.7 cells treated with different concentrations of baicalein.

Treatment group	Percent inhibition (%) ± S.E. of NO production	Percent inhibition (%) ± S.E. of PGE_2 production
Positive Control*	75.72 ± 2.52^{b}	99.72 ± 0.04^{b}
Vehicle Control	6.87 ± 3.15^{a}	3.59 ± 1.33^{a}
Baicalein (10 µM)	56.90 ± 1.77^{c}	48.46 ± 2.17^{c}
Baicalein (50 µM)	60.90 ± 1.26^{c}	73.87 ± 1.36^{d}
Baicalein (100 µM)	63.38 ± 2.04^{c}	87.37 ± 0.76^{e}

S.E. – Standard Error; LPS – Lipopolysaccharide; NO – Nitric Oxide; PGE_2 – Prostaglandin E_2
Means bearing different superscripts between treatment groups differ significantly ($P<0.01$). * Data of positive control were taken from our own published research (VARIA et al., 2020)

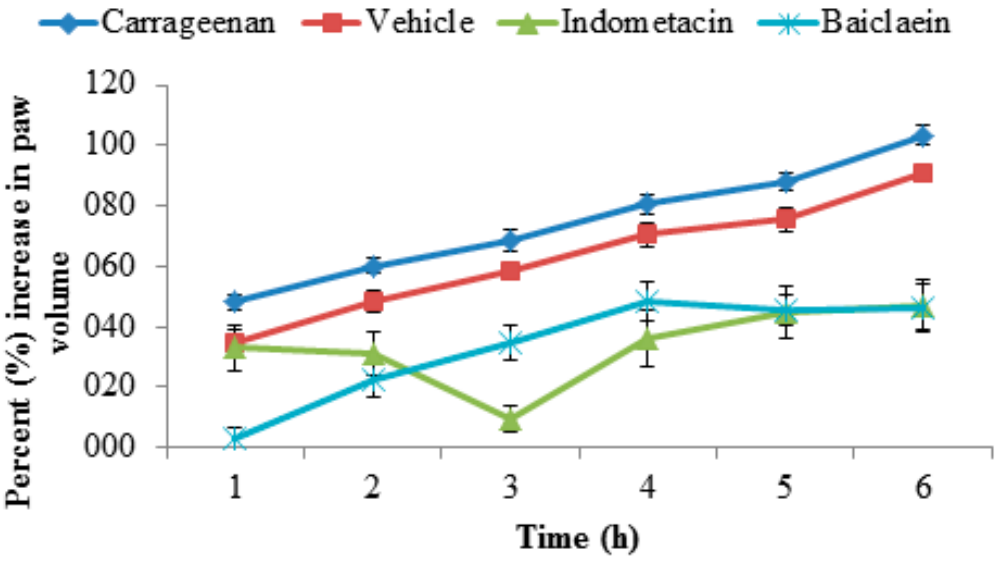

Fig. 1. Percentage increase (%) in paw volumes (Mean ± S.E.) of carrageenan induced inflammation in rats treated with baicalein and controls compared at 0 hour (n=6)

Table 2. Percentage inhibition (%) of paw volumes (Mean ± S.E.) of carrageenan induced inflammation in rats treated with drugs, compared with carrageenan control (n=6)

Group	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	6 Hours
Vehicle*	27.65 ± 8.83^{aA}	19.83 ± 5.90^{abB}	14.34 ± 3.73^{aC}	12.63 ± 5.24^{aD}	14.31 ± 4.55^{aD}	12.37 ± 2.80^{aE}
Indomethacin*	31.32 ± 15.35^{aA}	48.46 ± 1.73^{bAB}	86.18 ± 6.33^{bB}	54.80 ± 11.57^{bA}	48.90 ± 9.88^{bA}	54.54 ± 8.03^{bA}
Baicalein	93.65 ± 7.26^{bA}	62.41 ± 9.65^{bB}	49.29 ± 8.05^{cBC}	39.77 ± 7.82^{bC}	48.10 ± 5.82^{bC}	55.08 ± 7.71^{bC}

S.E. – Standard Error

Means bearing different superscripts in small letters between treatment groups and in capital letters within groups differ significantly ($P<0.01$). * Data of control groups were taken from our own published research (VARIA et al., 2020)

The *in vitro* anti-inflammatory results observed in the present study were in agreement with the results of other studies. Baicalein (5, 10, 20 and 40 μM) inhibited significant NO production in LPS-induced RAW 264.7 macrophage with an IC_{50} value of 19.4 ± 1.0 μM (CHEN et al., 2001), and baicalein (5, 10, 25 and 50 μM) was found to have an NO production inhibitory effect in a dose-dependent manner in an LPS-induced murine macrophage cell line (SEO et al., 2011). For PGE_2 production inhibition, a dose dependent effect was observed in the present study which was in line with the results obtained by KANEKO et al. (2010) with the IC_{50} value > 10 μM. In contrast to the results of the present study, CHEN et al. (2001) did not observe any inhibitory effect on PGE_2 production in RAW 264.7 macrophage. The results of the present *in vivo* experiment were in agreement with the results reported following subcutaneous administration of baicalein (20 mg/kg) and percentage inhibitions were observed as 24.3 %, 23.7 %, 27.7 % and 17.1 % after 1, 2, 3, and 4 hours in a carrageenan-induced rat paw oedema model, respectively (LIN and SHIEH, 1996). The present study and research by other authors demonstrate that baicalein has potential to inhibit the inflammatory reactions created by carrageenan in rats, which is reflected as the inhibition of inflammatory mediators, such as nitric oxide synthase enzyme, and others. Moreover, the anti-inflammatory action produced by baicalein may be due to inhibition of the major inflammatory enzyme cyclooxygenase-2, which is reflected as prostaglandins, i.e. PGE_2, in the present study. Contrary to the present study, the *in vivo* anti-inflammatory effect of *Scutellaria baicalensis* extract, which contains baicalein as a major active ingredient, exerted no significant inhibition on carrageenan-induced paw oedema (LEE et al., 2007).

Antibacterial effect: No minimum inhibitory concentrations of baicalein, against all the studied gram positive and gram negative organisms, were observed up to the maximum concentration studied *i.e.* 10.0 mg/ml. There was no *in vivo* antibacterial efficacy of baicalein observed using the neutropenic rat thigh infection (*Escherichia coli*) model. In agreement with the present study, KIM et al. (2014) observed no antibacterial activity of baicalein via the disc diffusion method against various pathogens, that is: *Escherichia coli* ATCC43889, *Escherichia coli* ATCC43890, *Escherichia coli* ATCC35150, *Psuedomonas aeruginosa* ATCC15692 and *Salmonella typhimurium* ATCC14028. However, compared to the present study, a lower MIC range value of baicalein against *Staphylococcus* spp. was reported using the broth dilution method at 32-64 μg/mL (PENG et al., 2011), and against *Streptococcus* spp. at 80-320 μg/mL (JANG et al., 2014). The reason behind the lack of antibacterial activity in the present study and reported by other authors may be due to the non compatibility of solvents used to dissolve baicalein for antibacterial

assay. Moreover, several authors observed the low systemic bioavailability of baicalein when administered alone, and this may be a major reason for the lack of *in vivo* antibacterial efficacy (TIAN et al., 2012; ZHU et al., 2017; LI et al., 2018). Although, the combination of baicalein with other synthetic antibiotics showed synergistic action against various bacteria (LEE et al., 2015; SIRIWONG et al., 2015; CAI et al., 2016). Therefore, it is suggested to use baicalein with standard antibacterials to increase their efficacy rather than as a sole agent. This synergism may be useful to combat bacterial resistance against various pathogens.

Pharmacokinetics of Baicalein: The results regarding the plasma levels and pharmacokinetics of baicalein are shown in Table 3, Table 4 and Fig. 2. A representative chromatogram of baicalein is also shown in Fig. 3. In the present study, the maximum baicalein plasma concentration (C_{max}) achieved was 0.77 ± 0.02 µg/mL at 0.08 h (T_{max}). A similar C_{max} (0.61 ± 0.32 µg/mL) was reported in monkeys at a dose of 500 mg/kg by TIAN et al. (2012). However, a lower maximal plasma concentration (C_{max}) was reported as 0.17 ± 0.07 µg/mL and 0.32 ± 0.05 µg/mL in monkeys at doses of 50 mg/kg and 150 mg/kg, respectively (TIAN et al., 2012), 0.11 µg/mL in humans (LI et al., 2014), and 0.0001 µg/mL in rats (KIM et al., 2007). In contrast, a higher C_{max} was detected as 21.13 µg/mL in dogs by TIAN et al. (2011). The peak baicalein plasma concentration achieved within 0.08 h in the present study indicates the quick absorption of the drug after intramuscular administration. The lower concentrations of baicalein detected may be due to the fact that baicalein, as a parent drug, is quickly and extensively converted to its 7-O-β-glucopyranuronoside metabolite of baicalein, indicating that most of the baicalein circulates as its conjugated metabolites (LAI et al., 2003; TIAN et al., 2012). The area under the plasma concentration curve (AUC) in the present study was detected as 0.30 ± 0.01 µg·h/mL, which is in line with AUC reported as 0.45 ± 0.12 µg·h/mL in monkeys at doses of 50 mg/kg (TIAN et al., 2012) and 0.54 µg·h/mL in humans (LI et al., 2014). Contrary to the present study, higher AUC values were reported as 1.16 ± 0.21 µg·h/mL at a dose of 150 mg/kg and 2.53 ± 1.54 µg·h/mL at a dose of 500 mg/kg in monkeys (TIAN et al., 2012) and 4.97 µg·h/mL in beagle dogs (TIAN et al., 2011). However, lower AUC values of 0.014 ± 0.01 µg·h/mL (LAI et al., 2003) and 0.0001 µg·h/mL (KIM et al., 2007) were reported in rats. The difference in AUC values may be due to variations in UDP-glucuronosyltransferase activity and metabolic rates in different species (WANG et al., 2010), as well as different dose rates.

Table 3. Plasma concentrations (µg/mL) of baicalein (200 mg/kg) following intramuscular administration in rats (n = 6)

Time after drug administration (h)	Plasma concentration (µg/mL) of baicalein
	Mean ± S.E.
0.03	0.53 ± 0.03
0.08	0.77 ± 0.02
0.17	0.35 ± 0.05
0.25	0.22 ± 0.01
0.33	0.17 ± 0.01
0.50	0.17 ± 0.01
0.75	0.11 ± 0.01
1.00	0.09 ± 0.01
2.00	ND

S.E. – Standard Error; ND: Not detected

Table 4. Pharmacokinetic parameters of baicalein (200 mg/kg) following intramuscular administration in rats (n = 6)

Pharmacokinetic parameters	Unit	Mean ± S.E.
α	h^{-1}	20.58 ± 7.55
β	h^{-1}	1.15 ± 0.11
$t_{1/2\alpha}$	h	0.37 ± 0.33
$t_{1/2\beta}$	h	0.63 ± 0.06
C_{max}	µg/mL	0.77 ± 0.02
T_{max}	h	0.08 ± 0.00
$AUC_{(0-\alpha)}$	µg.h/mL	0.30 ± 0.01
AUMC	$\mu g.h^2/mL$	0.23 ± 0.03
Vd_{area}	L/kg	601.03 ± 28.18
Cl_B	L/h/kg	677.39 ± 35.36
MRT	h	0.76 ± 0.06

α-distribution rate constant; β - elimination rate constant; $t_{1/2\alpha}$-distribution half life; $t_{1/2\beta}$- elimination half life; C_{max} - maximum plasma concentration; T_{max}- time at maximum plasma concentration achieved; $AUC_{(0-\alpha)}$- area under concentration; AUMC- area under the moment curve; Vd_{area}- volume of distribution; Cl_B- total body clearance; MRT - mean residence time; h^{-1} - per hour; h - hour; µg/mL - microgram per milliliter; µg.h/mL - microgram hour per milliliter; L/kg - liter per kilogram; L/h/kg - liter per hour per kilogram

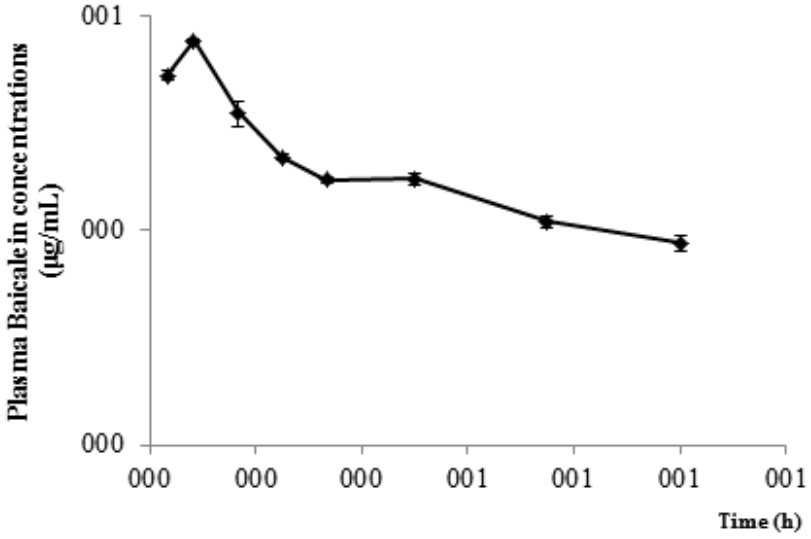

Fig. 2. Semi logarithmic plot of baicalein concentration in plasma versus time following single dose (200 mg/kg) intramuscular administration in rats. Each point represents mean ± S.E. (n = 6)

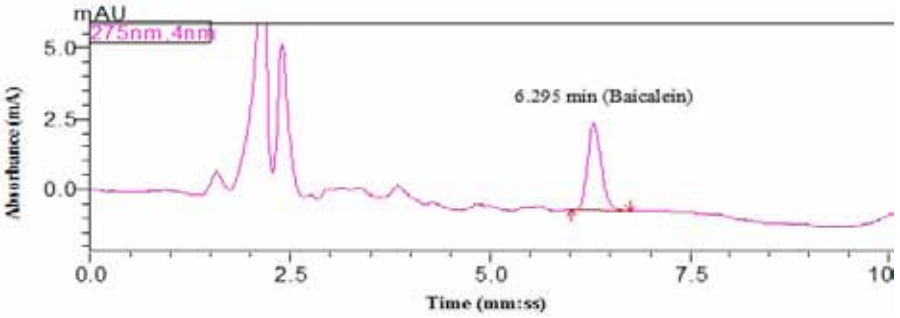

Fig. 3. Representative chromatogram 5 minutes post intramuscular administration of baicalein in rat at a dose rate of 200 mg/kg body weight.

In the present study, following single dose intramuscular administration of baicalein, the elimination half-life ($t_{1/2\beta}$) was 0.63 ± 0.06 h in rats, which is in line with 0.5 h in dogs after IV administration (TIAN et al., 2011). In contrast, higher elimination half-lives were reported, at 1.4 ± 1.0 h, 6.4 ± 3.6 h and 13.4 ± 9.5 h in monkeys, at 50, 150 and 500 mg/kg dose rates, respectively (TIAN et al., 2012), and 15.01 h in humans (LI et al., 2014). In the present study, high volumes of distribution (Vd_{area}) at 601.03 ± 28.18 L/h/kg and total body clearance (Cl) at 677.39 ± 35.36 L/kg were observed in rats, which were higher than those reported in beagle dogs following intravenous administration of baicalein at 1.73 L/h/kg (Vd_{area}) and 1.24 L/kg (Cl) (TIAN et al., 2011). The high volume of distribution of baicalein and lower C_{max} achieved after intramuscular administration may be due to its rapid absorption and distribution. In the present study, the mean residence time (MRT) was observed as 0.76 ± 0.06 h following intramuscular administration of baicalein in rats, which is in agreement with the MRT of 0.34 h reported in dogs after intravenous administration (TIAN et al., 2011). In contrast, higher MRTs of 2.9 ± 0.3 h, 5.9 ± 0.8 h and 6.2 ± 1.5 h were reported in monkeys at different dose levels (TIAN et al., 2012) and 10.86 ± 4.38 h in rats (LAI et al., 2003). As discussed earlier, due to rapid hepatic metabolism, the quick and extensive conversion of baicalein to its metabolite may be responsible for the high clearance rate of the drug, and this may lead to the lower elimination half-life and mean residence time (MRT).

Regarding its overall pharmacokinetic profile, baicalein should not be used as a conventional preparation since its maximum plasma concentration, elimination half-life and mean residence time are lower, which is not desirable. Therefore, different methodologies of administration or preparation, such as cocrystalisation, nano-particle encapsulation etc., should be tried and further research should be carried out in this regard.

Conclusions

Baicalein did not show *in vitro* and *in vivo* antibacterial activity. The *in vitro* anti-inflammatory activity of baicalein was found to inhibit NO production and PGE_2 production in a dose dependent manner in RAW 264.7 macrophage cells. A significant percentage inhibition of oedema volume was observed following intramuscular baicalein injection. Baicalein was found to have a high volume of distribution, with a lower C_{max}, short elimination half-life and mean residence time (MRT). Baicalein may be used in conjunction with a lower dose of traditional anti-inflammatory drugs to decrease side effects.

Acknowledgements

The authors express gratitude to the authorities of Veterinary College, Navsari Agricultural University, Navsari, Gujarat, for providing the opportunity and infrastructure facilities to carry out this research work.

Conflict of interest

All authors declare that they have no potential conflict of interest

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Received: 6. May 2021
Accepted: 26 November 2021.

VARIA R. D., J. H. PATEL, F. D. MODI, P. D. VIHOL, S. K. BHAVSAR: *In vitro* i *in vivo* protuupalna, antibakterijska i farmakokinetička svojstva baikaleina. Vet. arhiv 93, 117-128 2023.

SAŽETAK

Baikalein je bioaktivni flavon izvorno izoliran iz korijena biljaka *Scutellaria baicalensis, Scutellariala teriflora* i *Oroxylum indicum*. U ovom su radu istraživana *in vitro* i *in vivo* protuupalna i antibakterijska svojstva baikaleina te farmakokinetika nakon njegove pojedinačne intramuskularne primjene u *wistar* štakora. *In vitro* protuupalno djelovanje baikaleina (10, 50 i 100 µM) analizirano je s obzirom na sposobnost inhibicije enzima COX-2 mjerenjem razine PGE2 i određivanjem proizvodnje dušikova oksida (NO) u makrofagnim stanicama RAW 264,7 tretiranim lipopolisaharidom (LPS). Ustanovljeno je da baikalein znakovito inhibira proizvodnju NO i PGE2 u makrofagnim stanicama RAW 264,7 u usporedbi s LPS kontrolnom skupinom. *In vivo* protuupalno djelovanje baikaleina (200 mg/kg) procijenjeno je pomoću modela za mjerenje edema šape nakon intramuskularne injekcije karagenana, te je uočena znakovita inhibicija volumena edema u usporedbi s kontrolnom skupinom. *In vitro* i *in vivo* antibakterijsko djelovanje baikaleina određeno je metodom razrjeđivanja mikrobujona te modelom infekcije bedra neutropeničnog štakora, pri čemu baikalein nije pokazao antibakterijska svojstva. Koncentracije baikaleina utvrđene su u plazmi štakora tekućinskom kromatografijom visoke djelotvornosti (HPLC) nakon pojedinačne intramuskularne primjene u dozi od 200 mg/kg tjelesne mase u kojoj je prosječna vršna koncentracija lijeka (C_{max}) bila 0,77 ± 0,02 µg/mL, a postignuta je za 0,08 h. Prosječan poluživot eliminacije ($t_{½β}$) bio je 0,63 ± 0,06 h, providni volumen distribucije ($Vd_{(površina)}$) 601,03 ± 28,18 L/kg, ukupni tjelesni klirens ($Cl_{(B)}$) 677.39 ± 35.36 L/h/kg, a prosječno vrijeme zadržavanja (MRT) 0,76 ± 0,06 h. Zaključeno je da u ovom istraživanju baikalein nije pokazao *in vitro* i *in vivo* antibakterijska svojstva, ali je pokazao dobro protuupalno djelovanje. S obzirom na to da dostupni protuupalni lijekovi imaju nuspojave u liječenju ljudi i životinja, baikalein bi mogao biti učinkovita alternativa nesteroidnim protuupalnim lijekovima te bi ga trebalo istražiti i kod ciljanih životinjskih vrsta. Potrebna su daljnja istraživanja kojima bi se poboljšala topljivost i bioraspoloživost baikaleina injekcijskom primjenom.

Ključne riječi: antibakterijski; protuupalni; baikalein; farmakokinetika, štakor